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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G06K 9/00 — A1

(11) International Publication Number: WO 94/19767

(43) International Publication Date: 1 September 1994 (01.09.94)

(21) International Application Number: PCT/US94/01925

(22) International Filing Date: 24 February 1994 (24.02.94)

(30) Priority Data:
08/023,113 26 February 1993 (26.02.93) US

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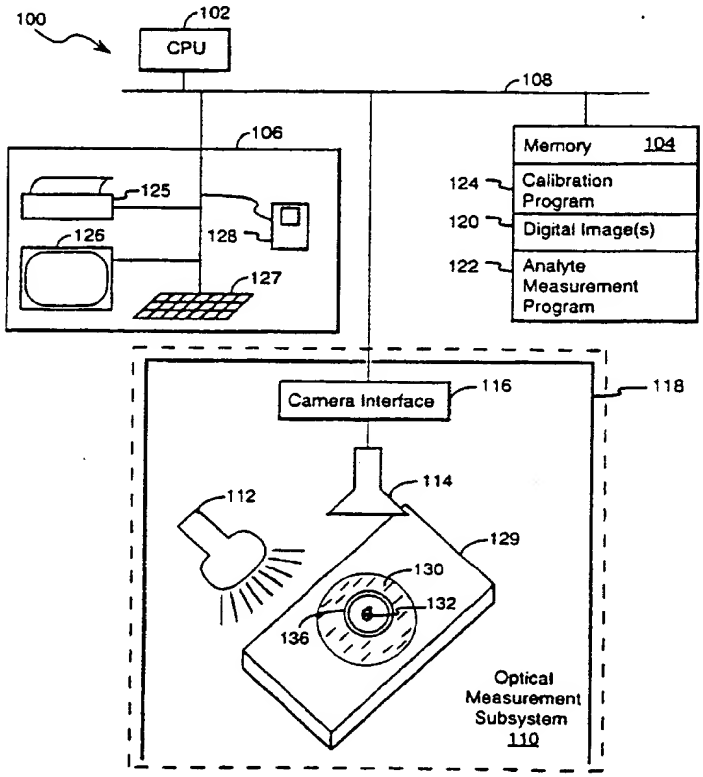
(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
With amended claims and statement.

(54) Title: OPTICAL SPECIMEN ANALYSIS SYSTEM AND METHOD

(57) Abstract

The present invention defines a method and system for automatically locating and analyzing an analyte deposited on the surface of a testing substrate containing a reagent. A positive result of the test will be visually noted as a darker region where the analyte has reacted with the reagent. Initially, the present invention calibrates the light required for determining the test results (124). A digital image is captured from the analog image (118) and processed (102) by the present invention. The present invention automatically locates the region of interest and computes an area of greatest optical or color density corresponding to the site of the analyte. A second area, away from the reaction site, is used to provide a background density reading. The background density is then used to adjust the analyte's density measurement in accordance with a predefined mathematical function to provide the quantitative results of the test (122).



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OPTICAL SPECIMEN ANALYSIS SYSTEM AND METHOD

The present invention relates generally to systems and methods of analyzing specimens, preferable specimens of biological origin, and particularly to computerized methods and systems for optical analysis of such specimens.

BACKGROUND OF THE INVENTION

Many present laboratory tests are determined by how a particular specimen reacts with a specific reagent. Often, these tests are qualitatively determined by visual inspection. For example, current home pregnancy tests can detect certain hormones in a woman's urine by coloring the testing substrate, thus indicating a potential pregnancy. For such tests, a technician can tell the results at a glance. However, with increasing demand for diagnostic testing, visual inspection by a human technician becomes a bottleneck. In addition, human technicians are error prone, especially when performing diagnostic tests that require quantitative measurements based on a specimen's color or optical density.

Consequently, a number of prior art systems have been designed to partially automate the process of determining test results. One approach taken is to digitize the image of the testing substrate and automatically analyze the image for test results. U.S. Patent No. 5,018,209 issued May 21, 1991 and U.S. Patent No. 5,008,185 issued April 16, 1991, both to Bacus, describe digital image processing methods and apparatus to analyze various features of cells being

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viewed on a slide under a microscope. Because the cells (or portions thereof) are randomly located on the slide, the technician and system work in an interactive fashion whereby the technician manually locates the cells on the slide that the system thereafter analyzes. Thus, while the efficiency of the testing process is increased by such an interactive system, it is not as efficient as one which would automatically locate the region of interest without human interaction.

U.S. Patent No. 4,922,915 issued May 8, 1990 to Arnold, describes an automatic image location method in the field of medical imaging technology, such as computer tomography (CT) and magnetic resonance imaging (MRI). In a typical diagnostic scan of a patient, several reference samples of known optical density are placed in proximity with the patient's body and are scanned simultaneously. These images of the reference samples of known density are compared with the images of various regions of the patient's body to determine the relevant characteristics of those regions.

The method in Arnold is concerned with locating two regions: the reference samples and the regions of interest within the patient's body. With respect to the reference samples, the system locates the samples automatically by two separate algorithms. The first algorithm uses the fact that the reference samples are of known optical densities. The Arnold system searches the entire digital image for regions with these optical densities.

The second location algorithm uses pre-positioned metallic rods proximately placed to the reference samples. Initially, the system starts scanning the entire digital image for pixels of greatest density. These pixels correspond to the metallic rods. Once the rods are located, the reference samples are easily located because the orientation of the samples in relation to the metallic rods is predefined.

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With respect to locating regions of interest in the patient's body, the search performed by the Arnold system is not fully automated. After the reference samples are located, Arnold requires that a human operator define an enlarged region of interest, for example around a bone structure, which the system thereafter refines. This step in Arnold is necessary because the system is unable to exclude regions which add error to the density readings.

While Arnold's method of automatically locating digital images works well when regions are either of known densities or known orientations, it is not satisfactory when the region of interest has neither known intensity or position. In Arnold's method, human interaction during the analysis step is always required.

In addition, the above mentioned methods do not preform quantitative analyses of specimens, but rather only located a region of interest based on optical density. However, in the analysis of chemical and biological specimens it is often the density of a particular color that is the relevant measurement parameter. For instance, certain colloidal reagents are used to generate a red spot when a biological specimen with a particular antigen is reacted with the colloidal reagent, thereby detecting the presence of the antigen in the biological specimen.

Therefore, it is an object of the present invention to provide a system and method for automatic image location and quantitative analysis when the region of interest is of neither known intensity or position in the image.

Another object of the present invention is to provide reliable automated test processing for laboratory tests in which the color saturation or optical density of a tested specimen must be quantified with reference to a predefined scale, which is more labor intensive than tests which yield a "yes/no" type of result. Furthermore, quantifying test results is very difficult for human technicians when the color density of the substrate surrounding the specimen is affected by the amount of reagent used for each test, by the amount of liquid (such as blood

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plasma) deposited on the substrate with the specimen, or by different batches of reagent. The results of such tests often depend on the difference in color saturation associated with the specimen and with the substrate region around the specimen (i.e., affected by the reagent). This is particularly true for tests using chemically active membranes as the substrates upon which tissue specimens are deposited.

SUMMARY OF THE INVENTION

In summary, the present invention is a method and system for the automatic analysis of an analyte derived from a specimen, such as a specimen of biological origin, when neither the position nor the optical density (or color density) of the region of interest is precisely known.

The present invention comprises the steps of depositing an analyte on a testing substrate in such a manner that at least a portion of the substrate is not covered by analyte. The substrate contains a pre-deposited reagent which reacts with analytes having certain predefined characteristics.

The substrate is illuminated and a digital image of the substrate is captured, preferably using reflected light. The illumination is pre-calibrated to correct for lighting intensity variations. Once captured, the digital image is automatically scanned and the most optically dense portion of the analyte is located. To aid in the scanning of the image, one embodiment of the substrate has a positional marker (either a dark or colored circle, line, spot, or any other shape) to generally indicate where the analyte was deposited. The use of a positional marker reduces both the amount of time require to locate the analyte and the degree of error in locating the densest portion of the analyte. After locating the region of greatest density, a measurement of its density is generated.

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A second region of the substrate not covered by the analyte is located and its density measurement is also generated. Because this second region is not proximate to the analyte, this region represents the background density of the substrate, which can be considered to be the background "noise" in the measurement of the analyte. The background density is then used to adjust the analyte's density measurement in accordance with a predefined mathematical function to provide the result of the test.

This test result may be further interpreted to determine the results of the particular test. In some cases, the result of the test could be interpreted as a binary, positive/negative result according to whether the measurement taken is above or below a given threshold. In other cases, the results may present a continuous range of values dependant upon a predefined function of the differential test output value.

BRIEF DESCRIPTION OF THE DRAWINGS

Additional objects and features of the invention will be more readily apparent from the following detailed description and appended claims when taken in conjunction with the drawings, in which:

Figure 1 is a block diagram of a system for optically analyzing biological and other analytes.

Figure 2 shows a sample holder with multiple analytes deposited thereon.

Figure 3 is a conceptual diagram of the method used to locate the optically densest portion of an analyte deposited on a substrate.

Figure 4 is a flow chart of the steps of the present invention.

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Figure 5 is a block diagram of an alternate embodiment of a system for analyzing biological and other analytes.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring to Figure 1, there is shown a block diagram of the system for optically analyzing biological and other analytes signed in accordance with the principles of the present invention. System 10 ludes a central processing unit (CPU) 102, computer memory 104, user inte e 106, system communication bus 108, and an optical measurement subsystem 110. The optical measurement subsystem 110 includes a shadowless (i.e., uniform) light source 112, camera 114, and camera interface 116. The optical measurement subsystem 110 is typically enclosed in a housing 118 so that optical images of analytes can be obtained under controlled optical conditions.

Memory 104, which will typically include both random access memory and secondary memory such as magnetic disk storage mechanisms, is sufficiently large enough to store a plurality of digital images 120, analyte measurement program 122, and a light source calibration program 124. Alternatively, programs 122 and 124 could be stored on Read Only Memory (ROM) chips. It should be noted that the specific memory devices used are not important to the operation of the present invention so long as they have sufficient capacity and operating speed to enable the optical image analysis tasks described below.

The user interface 106 will typically include at least one output communication device such as a printer 125 and/or monitor 126 for communicating the results of tests conducted by the system, and at least one input communication device such as keyboard 127 and/or mouse pointer device 128. Many other combinations of user interfaces could be used, and the specific interfaces shown in Figure 1 should not be construed as a limitation.

Before any substrates are analyzed, light source 112 is calibrated. Calibration is performed each time the system is powered on, and may need to be performed periodically if the system is kept on for long periods of time, because any changes in the light source's intensity could affect the result of the test. For example, if the measurement produced by the test is greater than a certain threshold value, the result of the test may be deemed positive. Otherwise, the test may be negative. These threshold values, stored as constants (or as a mathematical formula) in the analyte measurement program 112, are based upon an certain light intensity level. Without proper calibration, the possibility of false test results increases.

In the preferred embodiment, calibration is accomplished by calibration program 124 by measuring the light source's intensity with a light sensor (see light sensor 186 in Figure 5). In an alternate embodiment of the invention, the calibration program 124 takes as input an image of a calibration substrate. In the preferred embodiment a calibration substrate is a regular testing substrate without any analyte deposited on its surface. The intensity of light is measured according to the average density of the image of the calibration substrate. Then, a calibration coefficient is computed by dividing the average image density with a predefined standard value. All subsequent image density values are multiplied by this calibration coefficient.

When system 100 is ready for operation, camera 114 takes an analog image of at least a portion of the top surface of test carrier 129. The top surface of carrier 129, as depicted in Figure 1, includes testing substrate 130 on which is deposited a chemically active reagent. Prior to insertion of carrier into the optical measurement subsystem 110, an analyte 132, typically derived from a biological specimen, is deposited onto the region of the test carrier where substrate 130 is located. In the preferred embodiment, the chemical interaction of the analytes 132 with the chemically active substrate 130 typically causes the analyte 132 to be the optically densest portion of the substrate 130.

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Figure 2 shows a test carrier 129' with multiple analytes 144 deposited on its substrate 130. Positional marker 146 gives the relative position of substrate 130 on carrier 129'. As previously mentioned, marker 146 may be any shape or size sufficient to indicate the general position or location of the substrate 130 and/or the analyte(s) on the test carrier 129'.

The analog image generated by camera 114 is digitized by camera interface 116 and sent via bus 108 to memory 104 where the digital image is stored as an array of pixel values. In the presently preferred embodiment, the portion of the test carrier 129 captured by the camera 114 is 5/16" x 5/16" and is represented as a 170 x 170 array of pixel elements. Additionally, the presently preferred embodiment has the capability to process both color and gray scale images, with 8-bit pixels (256 gray scale levels) being used for gray scale images and 24-bit pixels (256^3 levels) being used for color images. It should be appreciated that the image could be formed from more or less pixel elements and more or less scale image levels to alter the image resolution and sensitivity. It will also be appreciated that other data structures for image storage are possible.

Note that for some analyte measurement tests the measured "density" of the analyte and background regions of the digital image will be the total optical density of a portion of the digital image, while for other analyte measurement tests the measured density will be the density of a particular color. That is, when the digital image is a color image each pixel will be represented by Red, Green and Blue (RGB) values, and the test measurements can be based on any one or predefined combination of the three RGB color values for the image's pixels. Thus, the term "density" in the discussions below concerns the density of a preselected optical characteristic of the digital image which is relevant to the measurement being performed.

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After the digital image has been captured, the image is analyzed by analyte measurement program 122. This analysis includes locating the analyte by locating within the digitized image a circular region of predefined size having the greatest optical density, locating a "background" region of the substrate 130 that is not covered by the analyte, and computing a test result by computing a predefined mathematical function of the average density of the background region and the average density of analyte region. The mathematical function may be as simple as subtracting the background density from the analyte density, or may be a considerable more complex function. In the preferred embodiment, the circular region of greatest optical density is sized to be small enough so as to be entirely covered the smallest anticipated analyte, and thus the average optical density of the circular region should be representative of the optical density of the chemically reacted analyte.

To accomplish this analysis, analyte measurement program 122 performs three main processes: pre-scan, fine-scan, and background density compensation. It will be appreciated that program 122 executes differently according to whether a positional marker is included on the test carrier 128 or not. Program 122 takes the digital image stored in memory 104 as input and begins a raster scan of the entire image. If a positional marker is present, program 122 then performs a raster scan across the image to locate the marker, which will typically be either the pixels of greatest density, or pixels of a particular color. From the orientation of the marker, program 122 will determine a smaller region of interest. Thereafter, program 122 confines its pre-scan and fine-scan processes to this defined region. If no positional marker is present on the substrate, then program 122 executes its pre-scan and fine-scan processes on a predefined region of the digital image.

The pre-scan process searches the region of interest for an area of greatest average density. This area will correspond to the reaction of the analyte to the reagent. The area should be as large as possible while still fitting entirely within the site of the analyte and should be sufficiently small to avoid noise sensitivity.

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In the preferred embodiment, the portion of the digital image used to determine the optical density of the analyte is a circle of diameter 64 pixels across.

Figure 3 is a conceptual diagram of the pre-scan process used to approximately locate the densest area of the analyte 132 deposited on the substrate 130. Please note that Figure 3 is not drawn to scale, and that the analyte 132 will typically cover a much smaller fraction of the substrate 130 than shown in this conceptual representation of the scanning process. The pre-scan process measures and compares the densities of a sequence of circular regions 160 arranged in columns and rows, where the centers of the columns are spaced apart by a distance of ΔX and the centers of the rows are spaced apart by a distance of ΔY. By comparing the densities of these regions the prescan process determines center of the circular region 160 of greatest density, as represented by circular region 162, and thereby locates the approximate center of the analyte. Table 1 contains a pseudocode representation of this process.

TABLE 1

PRESCAN PROCESS

```
        --      Specify range of regions to be tested:
                X1, Y1 = center of top-left region to be tested
                X2, Y2 = center of bottom-right region to be tested

        --      CX, CY and CD represent the position and density
        --      of the region of greatest optical density found so far.
                CX = X1
                CY = Y1
                CD = 0

                For X = X1 to X2, by steps of size ΔX
                {       For Y = Y1 to X2, by steps of size ΔY
                        {       Measure density D of region centered at X,Y within a radius
                                of Z pixels

                                --      Update center value whenever higher density region
                                --      is found

                                If D > CD
                                {       CX = X
                                        CY = Y
                                        CD = D
                                }
                        }
                }
```

After the region of greatest optical density is approximately found, the fine-scan process is used to more precisely locate the reaction region of greatest optical density. The fine-scan process takes as its input the center of the area of greatest density obtained from the prescan process. The center of the area is then shifted by one or two pixel elements in both the X and Y coordinates. The densities of these resulting areas are then calculated by summing the pixel element readings for the areas. The measured optical or color density for each are is compared with the greatest density located by the pre-scan process, and the greatest density area is accordingly updated. The fine-scan process then computes the average pixel density for the area by taking the greatest density reading and dividing by the number of pixels in the circle. This average density

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is the output result of the fine-scan procedure. Table 2 contains a pseudocode representation of this process.

TABLE 2

FINE-SCAN PROCESS

```
--    Initialize the starting X and Y ranges from the
--    center of the region found in Prescan.

      X_START = CX
      Y_START = CY

--    Let S1, S2, S3, S4, S5 and S6 be relatively small
--    integer values greater than zero.

      For X = X_START - S1 to X_START + S2, by steps of S3
      {  For Y = Y_START - S4 TO Y_START + S5, by steps of S6
            {     Measure density D of region centered at X,Y
                  within a radius of Z pixels.

                  --    Update center value whenever higher
                  --    density region is found.

                  If D > CD
                  {     CX = X
                        CY = Y
                        CD = D
                  }
            }
      }
```

After the fine-scan process refines the center coordinates of the region of greatest density, the analyte measurement program 122 then performs a background compensation step. To obtain the necessary background reading, annular region 136, as shown in Figure 1, is selected by program 122. Annular region 136 has an inner radius sufficiently large such that none of the analyte 132 is found in region 136. An average pixel density for annular region 136 is computed as discussed above. The measured density of the analyte is then adjusted in accordance with the measurement background region density. In some cases

the adjustment is simply a subtraction operation, while in others it may be accomplished by division or other mathematical operation.

This adjusted measurement value may be interpreted as the result of the test according to the nature of the reaction of the analyte with the reagent. In some cases, a simple threshold test will result. That is, if the adjusted measurement value is greater than a pre-determined threshold, then the test is considered positive. Otherwise, the test is considered negative. Alternatively, the adjusted measurement value may be a value in a continuous range of values that is to be interpreted by the user, or that is mapped with respect to a predefined scale and then presented for interpretation by the user.

The flow chart in Figure 4 represents the sequence of steps used to test an analyte, from depositing the analyte onto the substrate through generation of the final measurement value.

An alternate embodiment of the present invention is depicted in Figure 5. Instead of a positional marker being affixed to individual carriers, as shown as marker 146 in Figure 2, a separate template 180 is positioned between camera 114 and carrier 128. The image of template 180 is thus superimposed upon the image of carrier 128 when the image is captured. To insure the proper alignment of the two images, template 180 would remain in a fixed location; while carrier 128 would be slid into position by way of guide rails 182. The image of template 180 would be used to mark the region where the analyte is deposited on the substrate. Use of the template would obviate the need to place position markers on individual carriers.

Also depicted in Figure 5, a light sensor 186 can be positioned inside the measurement subsystem's housing 118 to measure the intensity of light emitted from light source 112. In this embodiment, readings from sensor 186 are used

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to calibrate the light source. This makes the calibration process totally automatic and thus the user is not required to perform or assist with the calibration process.

It will be appreciated that, although the presently preferred embodiment is currently used for the detection of antibodies to the human immunodeficiency virus (HIV), that application area is one of many potential applications and should not be construed as a limitation. In fact, the method and system of the present invention is broad enough to include the automatic testing of any analyte that visually reacts with any reagent.

It will further be appreciated that the present invention overcomes problems in prior automated systems. Specifically, the present invention does not require a human operator to work interactively at the analysis phase with the system to indicate the regions of interest for testing. Likewise, the present invention is able to locate the specific regions of interest without precisely knowing in advance either their location or their optical densities on the digital image.

While the present invention has been described with reference to a few specific embodiments, the description is illustrative of the invention and is not to be construed as limiting the invention. Various modifications may occur to those skilled in the art without departing from the true spirit and scope of the invention as defined by the appended claims.

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WHAT IS CLAIMED IS:

1. A method of analyzing an analyte, comprising the steps of:

(A) depositing said analyte on a substrate such that at least a portion of said substrate is not covered by said analyte;

(B) exposing said substrate to light;

(C) acquiring a digital image of said exposed substrate from light reflected by said analyte and substrate, said acquired image having at least two dimensions; and

(D) using data processing equipment,

(D1) automatically scanning said digital image, locating an optically densest portion of said analyte on said substrate, and generating a first measurement of the density of said densest portion;

(D2) locating a portion of said substrate not covered by said analyte, and generating a second measurement of the density of said uncovered portion of said substrate; and

(D3) generating an output signal by adjusting said first measurement with said second measurement in accordance with a predefined mathematical function.

2. The method of claim 1, wherein

said depositing step includes depositing a multiplicity of distinct analytes on distinct regions of said substrate;

said step (D) including locating and measuring the density of each analyte, and generating an output signal for each analyte.

3. The method of claim 1, wherein said substrate is opaque and includes a chemically active membrane on which said analyte is deposited.

4. The method of claim 3, wherein

said depositing step includes depositing a liquid on said membrane that changes said membrane's optical density in regions of said membrane not covered by said analyte;

said step D2 including locating a portion of said membrane near said analyte but not covered by said analyte, and generating said second measurement such that said second measurement is of the density of said uncovered portion of said membrane near said analyte.

5. The method of claim 1, wherein said substrate is opaque and includes a marker having a predefined spatial relationship to said analyte deposited on said substrate;

said step D1 including locating said marker in said digital image and then locating said analyte in said digital image based on said marker's location.

6. The method of claim 1, further including:

prior to step D, measuring optical density of a reference substrate and calibrating all subsequent measurements of substrates and analytes in accordance with said reference substrates measured optical density.

7. The method of claim 1, wherein

said step D2 further includes reading the optical density of said uncovered portion and calibrating the reading of said densest portion in accordance with said reading.

8. The method of claim 1, wherein said digital image comprises an N x M array of pixels; said step D1 including:

for each of a selected set of pixel positions spaced apart from each other, measuring the optical density of a region of said digital image associated with said each pixel position; and

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selecting a plurality of said measured regions with highest density, interpolating the pixel positions associated with said selected measured regions to generate a final pixel position, and then measuring the optical density of a region of said digital image associated with said final pixel position to generate said first measurement.

9. A system for anaiyzing an anaiyte derived from a specimen deposited on a substrate so that a portion of said substrate is not covered by said analyte, comprising:

means for acquiring a digital image of said substrate; and

data processing means, coupled to said image acquiring means, for automatically scanning said digital image to locate an optically densest portion digital image depicting said analyte and to locate a background portion of said digital image not depicting said analyte;

said data processing means including means for (A) generating a first measurement of the density of said densest portion, (B) generating a second measurement of the density of said background portion of said substrate, and (C) generating an output signal by adjusting said first measurement with said second measurement in accordance with a predefined mathematical function.

10. The system, as defined in claim 9, further including:

means of illuminating said substrate; and

means for sensing the intensity of said means for illumination;

said data processing means including means, coupled to said intensity sensing means, for calibrating said measurements in accordance with said sensed intensity of said means for illumination.

11. The system, as defined in claim 9, wherein:

a multiplicity of distinct analytes are deposited on distinct regions of said substrate;

said data processing means including means for locating an optically densest portion of each said distinct analyte, and for generating a first measurement of the density of said densest portions of each said distinct analyte.

12. The system, as defined in claim 9, wherein said substrate includes a marker having a predefined spatial relationship to said analyte; and

said data processing means includes means for locating said marker in said digital image and for locating said analyte within said digital image based on said marker's location.

13. The system, as defined in claim 9, further including:

a template interposed between said substrate and said means for acquiring a digital image, said template forming a superimposed image on said digitized image of said substrate;

a plurality of guide rails sized to receive said substrate so that said image of said template falls at predefined position with respect to said substrate, thereby indicating where said analyte is deposited on said substrate.

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14. A method of analyzing an analyte, comprising the steps of:

(A) depositing said analyte on a substrate such that at least a portion of said substrate is not covered by said analyte;

(B) uniformly exposing said substrate to light;

(C) acquiring a digital image of said exposed substrate, said acquired image having at least two dimensions; and

(D) using data processing equipment,

(D1) automatically scanning said digital image, locating an portion of said analyte on said substrate having greatest density of a predefined color, and generating a first measurement of the density of said predefined color at said located portion;

(D2) locating a portion of said substrate not covered by said analyte, and generating a second measurement of the density of said predefined color at said uncovered portion of said substrate; and

(D3) generating an output signal by adjusting said first measurement with said second measurement in accordance with a predefined mathematical function.

15. The method of claim 14, wherein

said depositing step includes depositing a multiplicity of distinct analytes on distinct regions of said substrate;

said step (D) including locating and measuring the density of said predefined color for each analyte, and generating an output signal for each analyte.

16. The method of claim 14, wherein said substrate is opaque and includes a chemically active membrane on which said analyte is deposited.

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17. The method of claim 16, wherein

said depositing step includes depositing a liquid on said membrane that changes said membrane's color density in regions of said membrane not covered by said analyte;

said step D2 including locating a portion of said membrane near said analyte but not covered by said analyte, and generating said second measurement such that said second measurement is of the density of said predefined color in said uncovered region of said membrane near said analyte.

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AMENDED CLAIMS

[received by the International Bureau on 5 July 1994 (05.07.94); original claims 1-17 replaced by amended claims 1-17 (6 pages)]

1. A method of analyzing an analyte of biological origin, comprising the steps of:

(A) providing a substrate having a chemically active reagent thereon, and depositing said analyte on said substrate such that at least a portion of said substrate is not covered by said analyte; wherein said analyte reacts, or does not react, with said reagent depending on associated characteristics of said analyte;

(B) exposing said substrate to light;

(C) acquiring a digital image of said exposed substrate from light reflected by said analyte and substrate, said acquired image having at least two dimensions; and

(D) using data processing equipment,

(D1) automatically scanning said digital image, locating an optically densest portion of said analyte on said substrate, and generating a first measurement of the density of said densest portion;

(D2) locating a portion of said substrate not covered by said analyte, and generating a second measurement of the density of said uncovered portion of said substrate; and

(D3) generating an output signal by adjusting said first measurement with said second measurement in accordance with a predefined mathematical function, said output signal representing a result of said analyte reacting or not reacting with said reagent.

2. The method of claim 1, wherein

said depositing step includes depositing a multiplicity of distinct analytes on distinct regions of said substrate;

said step (D) including locating and measuring the density of each analyte, and generating an output signal for each analyte.

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3. The method of claim 1, wherein said substrate is opaque and includes a chemically active membrane on which said analyte is deposited.

4. The method of claim 3, wherein

said depositing step includes depositing a liquid on said membrane that changes said membrane's optical density in regions of said membrane not covered by said analyte;

said step D2 including locating a portion of said membrane near said analyte but not covered by said analyte, and generating said second measurement such that said second measurement is of the density of said uncovered portion of said membrane near said analyte.

5. The method of claim 1, wherein said substrate is opaque and includes a marker having a predefined spatial relationship to said analyte deposited on said substrate;

said step D1 including locating said marker in said digital image and then locating said analyte in said digital image based on said marker's location.

6. The method of claim 1, further including:

prior to step D, measuring optical density of a reference substrate and calibrating all subsequent measurements of substrates and analytes in accordance with said reference substrates measured optical density.

7. The method of claim 1, wherein

said step D2 further includes reading the optical density of said uncovered portion and calibrating the reading of said densest portion in accordance with said reading.

8. The method of claim 1, wherein said digital image comprises an N x M array of pixels; said step D1 including:

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for each of a selected set of pixel positions spaced apart from each other, measuring the optical density of a region of said digital image associated with said each pixel position; and

selecting a plurality of said measured regions with highest density, interpolating the pixel positions associated with said selected measured regions to generate a final pixel position, and then measuring the optical density of a region of said digital image associated with said final pixel position to generate said first measurement.

9. A system for analyzing an analyte derived from a specimen of biological origin deposited on a substrate so that a portion of said substrate is not covered by said analyte, said substrate having a chemically active reagent thereon, wherein said analyte reacts, or does not react, with said reagent depending on associated characteristics of said analyte; said system comprising:

means for acquiring a digital image of said substrate; and

data processing means, coupled to said image acquiring means, for automatically scanning said digital image to locate an optically densest portion digital image depicting said analyte and to locate a background portion of said digital image not depicting said analyte;

wherein said data processing means is adapted to (A) generate a first measurement of the density of said densest portion, (B) generate a second measurement of the density of said background portion of said substrate, and (C) generate an output signal by adjusting said first measurement with said second measurement in accordance with a predefined mathematical function said output signal representing a result of said analyte reacting or not reacting with said reagent.

10. The system, as defined in claim 9, further including:

a light source that said substrate; and

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a light sensor that senses the intensity of said means for illumination;

said data processing means including means, coupled to said intensity sensing means, for calibrating said measurements in accordance with said sensed intensity of said means for illumination.

11. The system, as defined in claim 9, wherein:

a multiplicity of distinct analytes are deposited on distinct regions of said substrate;

said data processing means including means for locating an optically densest portion of each said distinct analyte, and for generating a first measurement of the density of said densest portions of each said distinct analyte.

12. The system, as defined in claim 9, wherein said substrate includes a marker having a predefined spatial relationship to said analyte; and

said data processing means includes means for locating said marker in said digital image and for locating said analyte within said digital image based on said marker's location.

13. The system, as defined in claim 9, further including:

a template interposed between said substrate and said means for acquiring a digital image, said template forming a superimposed image on said digitized image of said substrate;

a plurality of guide rails sized to receive said substrate so that said image of said template falls at predefined position with respect to said substrate, thereby indicating where said analyte is deposited on said substrate.

14. A method of analyzing an analyte of biological origin, comprising the steps of:

(A) providing a substrate having a chemically active reagent thereon,

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and depositing said analyte on said substrate such that at least a portion of said substrate is not covered by said analyte; wherein said analyte reacts, or does not react, with said reagent depending on associated characteristics of said analyte;

(B) uniformly exposing said substrate to light;

(C) acquiring a digital image of said exposed substrate, said acquired image having at least two dimensions; and

(D) using data processing equipment,

(D1) automatically scanning said digital image, locating an portion of said analyte on said substrate having greatest density of a predefined color, and generating a first measurement of the density of said predefined color at said located portion;

(D2) locating a portion of said substrate not covered by said analyte, and generating a second measurement of the density of said predefined color at said uncovered portion of said substrate; and

(D3) generating an output signal by adjusting said first measurement with said second measurement in accordance with a predefined mathematical function said output signal representing a result of said analyte reacting or not reacting with said reagent.

15. The method of claim 14, wherein

said depositing step includes depositing a multiplicity of distinct analytes on distinct regions of said substrate;

said step (D) including locating and measuring the density of said predefined color for each analyte, and generating an output signal for each analyte.

16. The method of claim 14, wherein said substrate is opaque and includes a chemically active membrane on which said analyte is deposited.

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17. The method of claim 16, wherein

said depositing step includes depositing a liquid on said membrane that changes said membrane's color density in regions of said membrane not covered by said analyte;

said step D2 including locating a portion of said membrane near said analyte but not covered by said analyte, and generating said second measurement such that said second measurement is of the density of said predefined color in said uncovered region of said membrane near said analyte.

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STATEMENT UNDER ARTICLE 19

All the pending claims are directed to a measurement apparatus and method, for measuring or analyzing the result of a chemical reaction. The present invention is an apparatus and method that measures the optical density of a physical sample (i.e., the analyte) after it has been exposed to a reagent.

The Deindoerfer and two Bacus references are both designed to either count or quantify things such as "labels" or cells. Deindoerfer determines the "number and location of the labels", while Bacus '845 classifies abnormal and normal red blood cells by analyzing the shape, size, and presence/absence of central pallor of interior cells structures. Bacus '185 uses a staining technique, but then uses color separations and the like to locate and analyze cells and portions of cells.

The present invention is does not locate and count things, and does not analyze interior cell structures. Rather, the present invention is directed to a system and method for measuring the result of exposing an analyte to a reagent, and is particularly directed to performing such measurements in situations where the background optical density is variable from sample to sample and a differential measurement is required.

While it might be possible to reprogram the Deindoerfer and Bacus systems to use the methodology of the present invention, the prior art of record does not address the problem solved by the present invention and does not teach or suggest the methodology and apparatus configuration used by the present invention.

The applicant wishes to point out that simplicity of structure does **not** equate to "lack of novel step". One first must recognize the true nature of a problem before one can propose its solution. The present invention provides an efficient and cost effective solution to a problem: the uneven results generated by human technicians when performing analyte reaction measurements in which the background optical density is either not uniform or not known in advance and must be factored out of the measurement being made.

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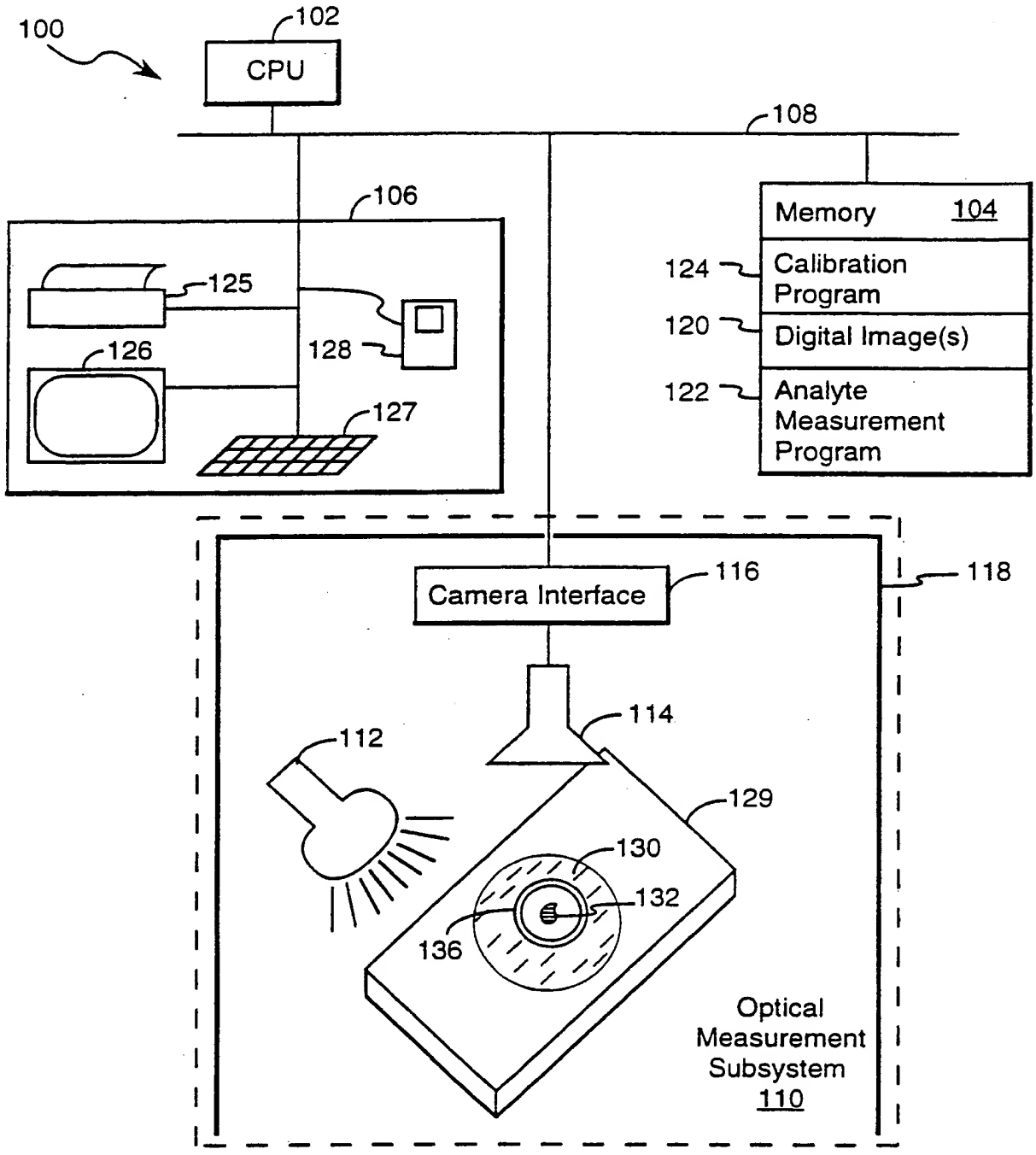

FIGURE 1

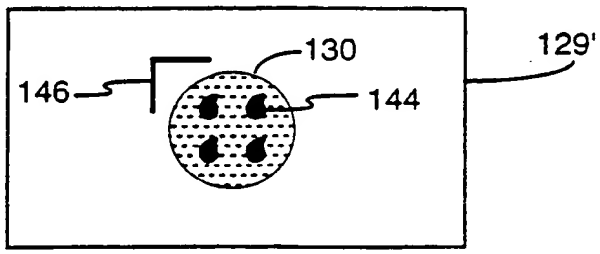

FIGURE 2

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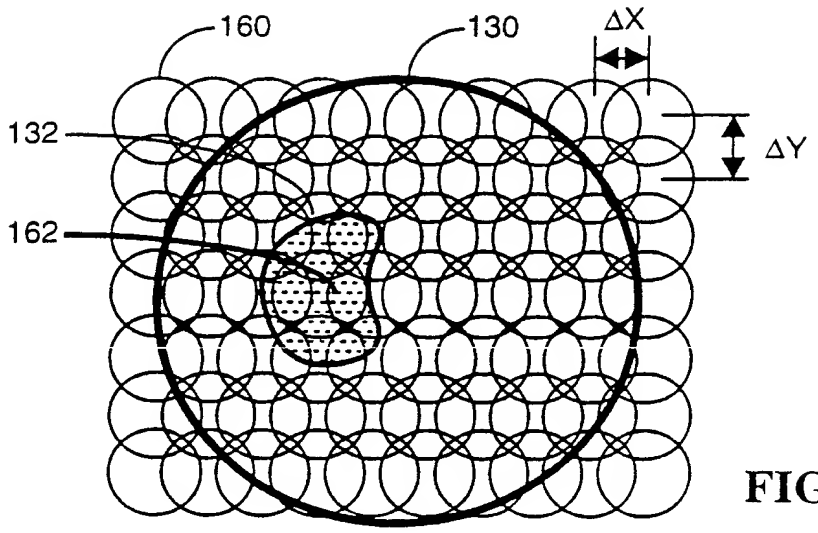

FIGURE 3

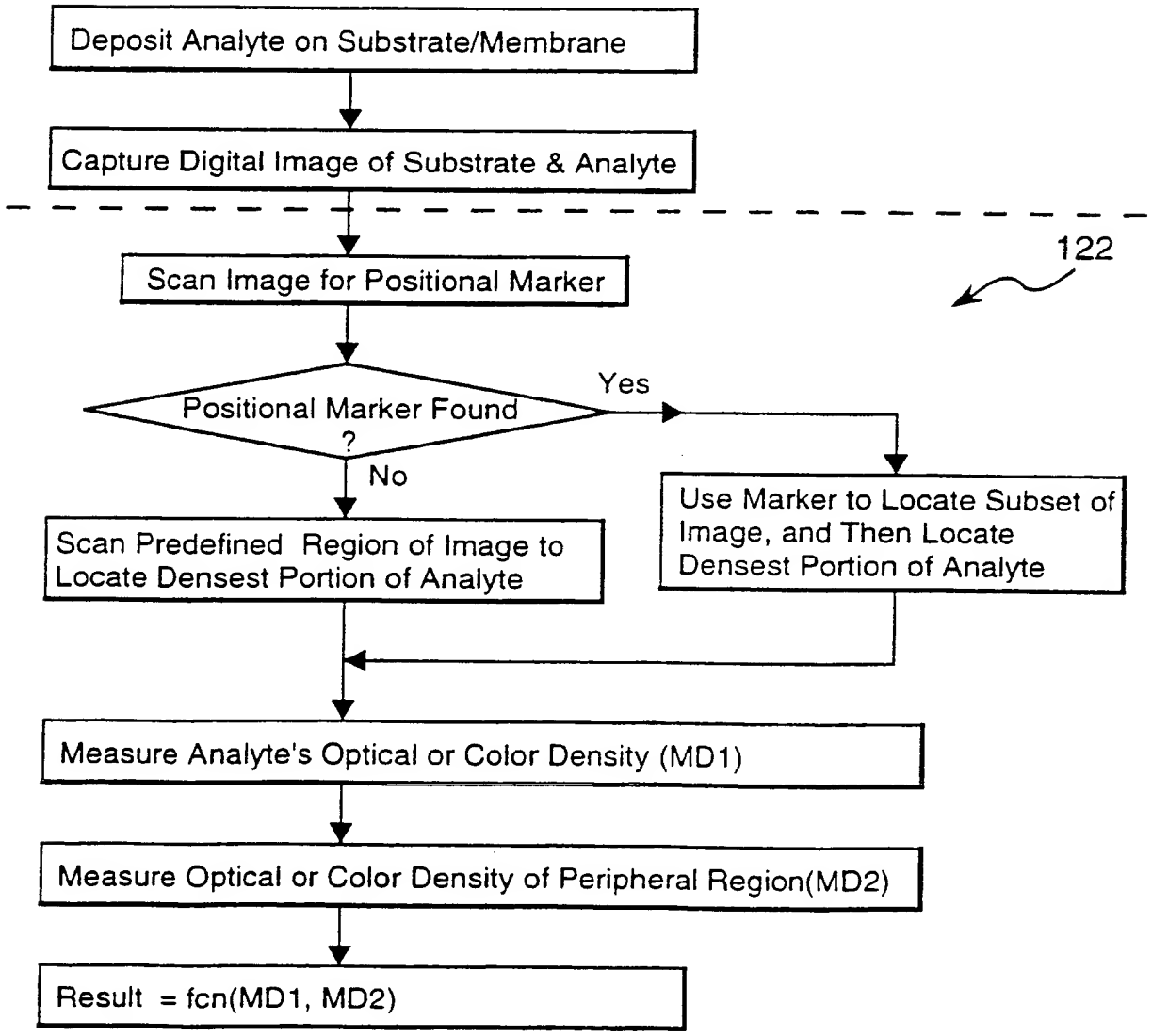

FIGURE 4

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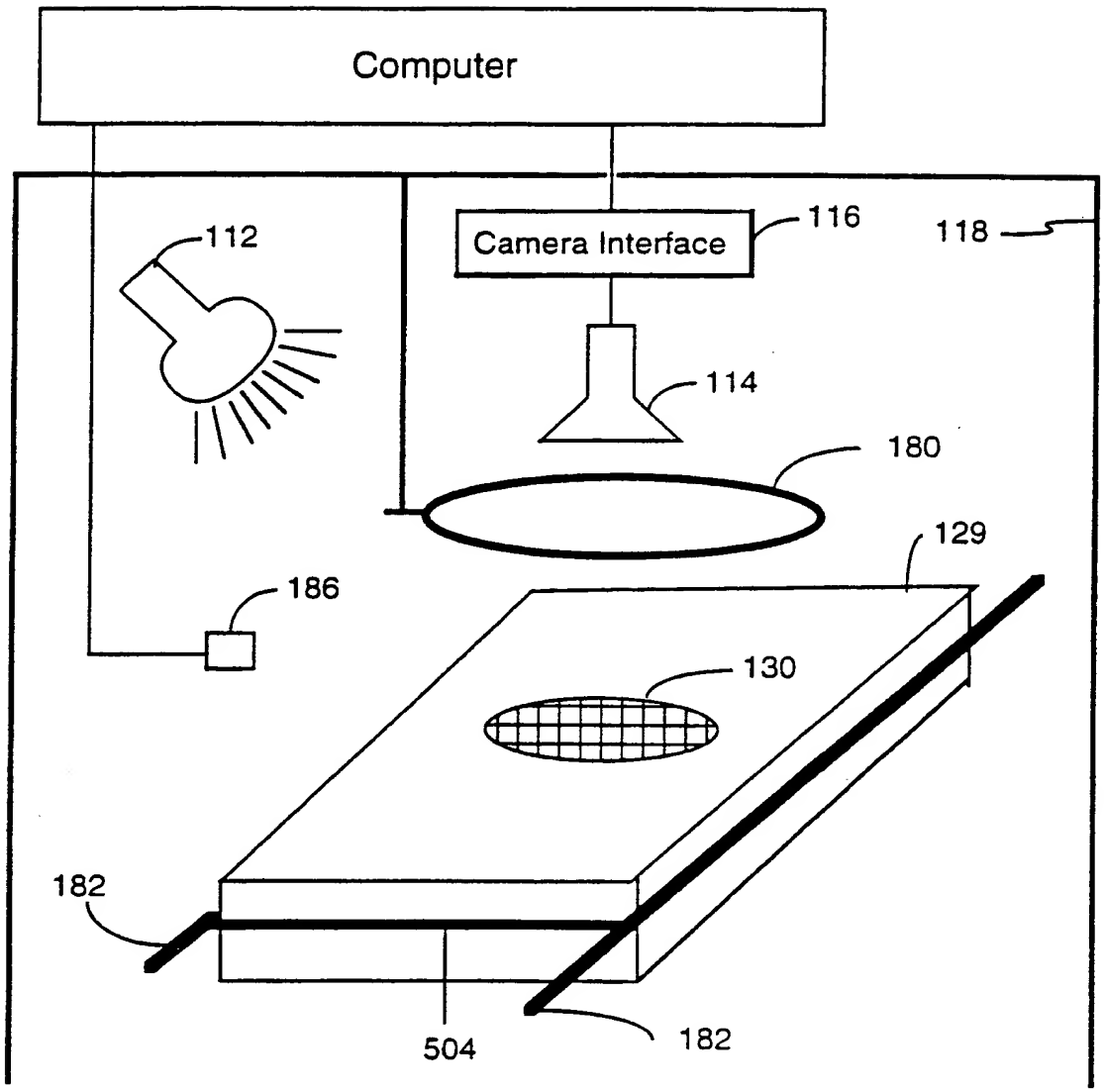

FIGURE 5

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INTERNATIONAL SEARCH REPORT

Inte . ional application No.
PCT/US94/01925

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :GO6K 9/00
US CL :382/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 382/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,476,231 (DEINDOERFER ET AL.) 09 October 1984, figure 1, and column 1, lines 32-46, column 3, lines 24-41, and column 4, lines 20-25.	1-17
Y	US, A, 4,097,845 (BACUS) 27 June 1978, figures 1-2 and 4-5, column 3, line 58 through column 4, line 15, and column 5, line 5 through column 8, line 4.	1, 3, 9-10, 14 and 16
Y	US, A, 5,008,185 (BACUS) 16 April 1991, figure 3, column 5, lines 20-55, column 10, lines 1-16, column 11, lines 14-60, column 12, lines 10-25 and column 13, lines 40-67.	2, 4-8, 11-13, 15 and 17

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
"E" earlier document published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
04 April 1994

Date of mailing of the international search report
MAY 11 1994

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,476,231 (DEINDORFER ET AL.) 09 October 1984, figure 1, and column 1, lines 32-46, column 3, lines 24-41 and column 4, lines 20-25.	1-17
Y	US, A, 4,097,845 (BACUS) 27 June 1978, figure 1-2 and 4-5, column 3, line 58 through column 4, line 15 and column 5, line 5 through column 8, line 4.	1, 3, 9-10, 14 and 16
Y	US, A, 5,008,185 (BACUS) 16 April 1991, figure 3, column 5, lines 20-55, column 10, lines 1-16, column 11, lines 14-60, column 12, lines 10-25 and column 13, lines 40-67.	2, 4-8, 11-13, 15 and 17

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